Simultaneous Labeling of Two Proteins in Live Cells

Introduction

Protein synthesis, transport, and degradation are highly dynamic processes. Expression, degradation and localization of a given protein can be rapidly modulated during a variety of cellular processes, including cell cycle progression, differentiation and apoptosis. Though there are a number of approaches for monitoring these processes indirectly in fixed cells and cell lysates, tools to study these events in real time are more limited.

SNAP- and CLIP-tags are novel tools for protein research, allowing the specific, covalent attachment of virtually any molecule to a protein of interest. The SNAP-tag® is based on mammalian O^6 -alkyl-guanine-DNA-alkyltransferase (AGT), a protein engineered to attach derivatives of benyzylpurine or benzylpyrimidines, including benzylguanine (BG) to itself. CLIP-tag™ is a modified version of SNAP-tag, engineered to react with benzylcytosine (BC) rather than benzylguanine derivatives, enabling the simultaneous labeling of two proteins in live cells (Figure 1) (1,2).

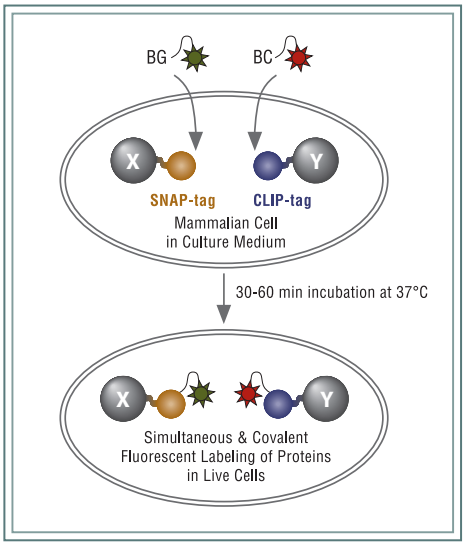

Figure 1: *Schematic of in vivo protein co-labeling with SNAP- and CLIP-tags. Benzylguanine (BG) and benzylcytosine (BC) fluorescent labels added to the culture media react with the proteins of interest (X and Y) fused to the SNAP-tag or CLIP-tag, respectively.*

Dual Protein Labeling in Live Cells

The ability to simultaneously label SNAP- and CLIP-tag fusion proteins provides researchers with a unique tool to explore protein dynamics in living cells. Figure 2 shows the concomitant labeling of two proteins in live cells: MEK1, a cytosolic protein kinase, and histone H2B, a nuclear protein. Briefly, MEK1 was fused to the C-terminus of CLIP-tag (CLIP-MEK1) and histone H2B was fused to the N-terminus of SNAP-tag (H2B-SNAP). CHO-K1 cells transfected with either or both fusion proteins then labeled with fluorescent substrates specific for SNAP-tag and CLIP-tag, SNAP-Cell® 505 (NEB #S9103) and CLIP-Cell™ TMR Star (NEB #S9219), respectively. Excess substrate was washed away and cells were observed by fluorescence microscopy. CLIP-MEK1 or H2B-SNAP exhibited the expected localization to the cytosol and nucleus, respectively, demonstrating that SNAP- and CLIP-tag labeling work efficiently in live cells (Figure 2). Labeling can be performed simultaneously (Figure 2A) or sequentially (Figure 2B, 2C). This experiment shows clear and specific compartmental labeling of MEK1 and H2B, demonstrating that the orthogonal tagging systems can be applied to the simultaneous labeling of two proteins inside the same cell.

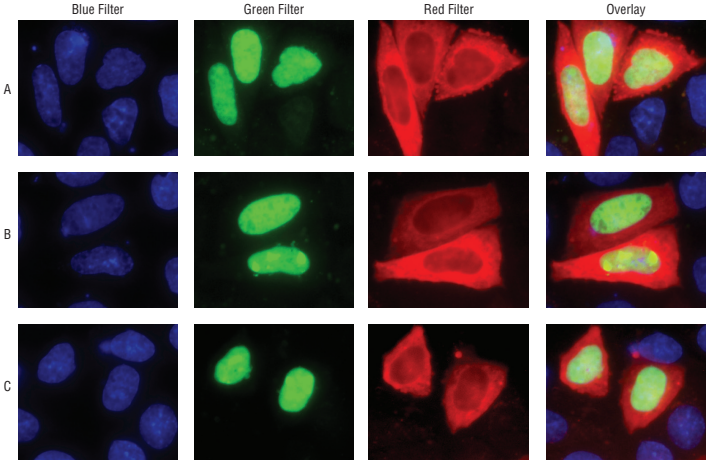

Figure 2: *Live cell fluorescent image of MEK1 and histone H2B tagged with CLIP-tag and SNAP-tag, respectively. CHO-K1 cells expressing both fusion proteins were incubated with 5 µM of the membrane permeable substrate SNAP-Cell 505 (green) and CLIP-Cell TMR Star (red), washed and imaged. Nuclei were counterstained with Hoechst dye (blue). Results are consistent when cells are labeled simultaneously (A) or sequentially, with CLIP-Cell TMR Star followed by SNAP-Cell 505 (B) or SNAP-Cell 505 followed by CLIP-Cell TMR Star (C).*

(See other side)

In a second example, MEK1 and ERK2, two protein kinases in the mitogen-activated protein kinase cascade that interact physically with one another, were used as a model system to examine the dynamic co-localization of two proteins in live cells. When expressed separately in mammalian cells, ERK2 is localized mostly in the nucleus while MEK1 is localized in the cytosol. However, when co-expressed in the same cell, ERK2 is sequestered to the cytosol (3). Figure 3 shows the expression and localization of the ERK2-CLIP and SNAP-MEK1 in live CHO-K1 cells. As expected, ERK2-CLIP is present in the nucleus when expressed alone, while co-expression of SNAP-MEK1 triggers the shuttling of ERK2-CLIP to the cytosol. These data illustrate that SNAP- and CLIP-tags are ideally suited to the simultaneous labeling of two proteins in live cells. Such analysis can be readily extended to a wide variety of other proteins. Furthermore, this image highlights the specificity of CLIP-Cell TMR-Star for ERK2-CLIP and SNAP-Cell 360 for MEK1-SNAP.

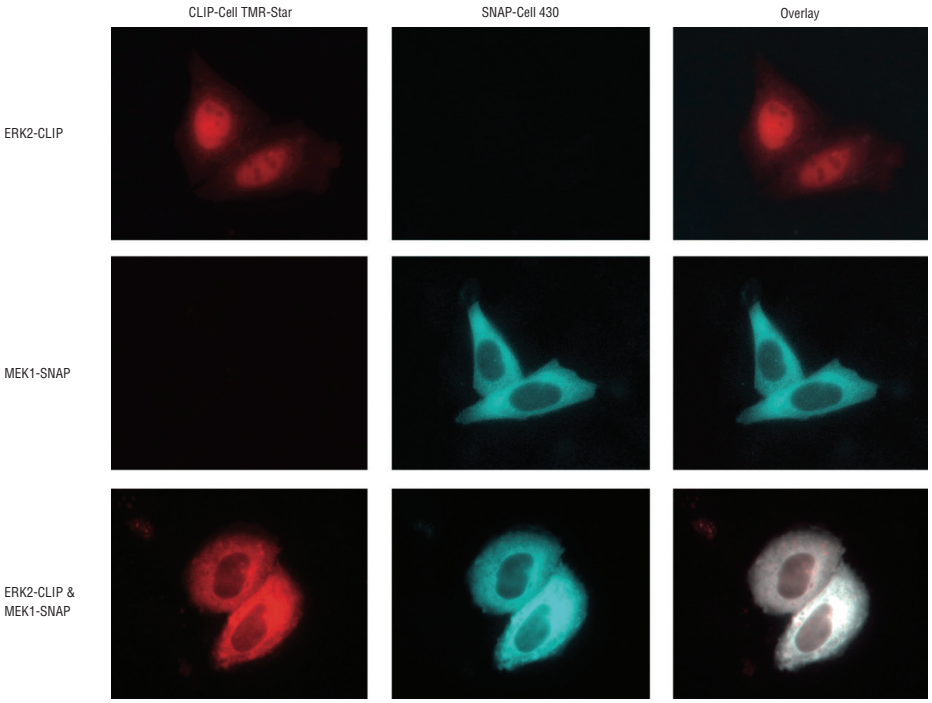

Figure 3: *Shuttling of ERK2 from the nucleus to the cytoplasm when co-expressed with MEK1 in live CHO-K1 cells. ERK2-CLIP was expressed alone or in the presence of SNAP-MEK1, cells were subsequently labeled with CLIP-Cell, TMR Star, SNAP-Cell 430 or both. All cells were treated with both CLIP-Cell TMR-Star and SNAP-Cell 430, but imaged using filters for visualizing each fluorophore independently.*

Summary

CLIP- and SNAP-tag are valuable tools for the selective labeling of proteins in live cells. As both tags have been engineered to react with distinct classes of substrates, the system can simultaneously visualize two proteins in the same cellular environment. The dynamics of their interaction, localization and stability can be readily examined following a variety of cellular stimuli.

General Protocol

Co-labeling of SNAP-tag and CLIP-tag fusion proteins

1. Seed cells in a plate (or chamber slide) designed for visualization via fluorescence microscopy.
2. Co-transfect the cells with a construct expressing a SNAP-tag fusion protein and a construct expressing a CLIP-tag fusion protein.
3. Incubate for 18–24 hours at 37°C in a cell culture incubator.
4. Wash the cells once with 1X complete medium and replace with a labeling mix containing 1 to 5 µM SNAP-Cell* label and 5 µM CLIP-Cell label diluted in prewarmed complete medium. (Medium can also contain regular Penicillin-Streptomycin antibiotic mix).
5. Incubate for 30 min to 1 hour at 37°C in a cell culture incubator.
6. Wash cells 3X with prewarmed complete medium then incubate them in fresh medium for 30 minutes. Replace the medium one more time to remove non-reacted substrates that have diffused out of the cells.
7. Image the cells using appropriate filter sets. Note: Nuclear staining can be performed by directly adding the Hoescht dye at 1X final concentration to the labeling mix 15 to 30 min before the end of the incubation.

*For SNAP-Cell TMR Star use 1 µM final concentration.

References:

1. Gautier A., et al. (2008) *Chem. Biol.*, 2, 128–136.
2. Schultz C. and Köhn M. (2008) *Chem. Biol.*, 2, 91–92.
3. Rubinfeld H., et al. (1999) *J. Bio. Chem.*, 274, 30349–30352.

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